DEPENDENCE OF EARLY AND LATE CHROMOSOMAL ABERRATIONS ON RADIATION QUALITY AND CELL TYPES

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Introduction

Exposure to radiation induces different types of DNA damage, increases mutation and chromosome aberration rates, and increases cellular transformation in vitro and in vivo. The susceptibility of cells to radiation depends on genetic background and growth condition of cells, as well as types of radiation. Mammalian cells of different tissue types and with different genetic background are known to have different survival rate and different mutation rate after cytogenetic insults. Genomic instability, induced by various genetic, metabolic, and environmental factors including radiation, is the driving force of tumorigenesis. Accurate measurements of the relative biological effectiveness (RBE) is important for estimating radiation-related risks.

To further understand genomic instability induced by charged particles and their RBE, we exposed human lymphocytes ex vivo, human fibroblast AG1522, human mammary epithelial cells (CH184B5F5/M10), and bone marrow cells isolated from CBA/CaH (CBA) and C57BL/6 (C57) mice to high energy protons and Fe ions. Normal human fibroblasts AG1522 have apparently normal DNA damage response and repair mechanisms, while mammary epithelial cells (M10) are deficient in the repair of DNA DSBs. Mouse strain CBA is radio-sensitive while C57 is radio-resistant. Metaphase chromosomes at different cell divisions after radiation exposure were collected and chromosome aberrations were analyzed as RBE for different cell lines exposed to different radiations at various time points up to one month post irradiation.

Materials and Methods

Peripheral whole blood from two healthy donors was collected in Vacutainer tubes containing sodium citrate. Peripheral blood mononuclear cells (PBMCs) were immediately separated by centrifugation, washed twice with PBS, counted and resuspended in RPMI1640 with 2mM Glutamine and10%FBS. Normal human fibroblasts AG1522 were grown in alpha-MEM with 10% FBS and antibiotics in humidified tissue culture chamber at 37˚C with 5% CO_2. Human mammary epithelial cells (CH184B5F5/M10) were cultured in DMEM medium with supplement of 10% FBS and antibiotics. Cells were exposed in vitro to Fe ions or protons (600MeV/nucleon) at NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory.

After irradiation, PBMCs were stimulated to grow in medium containing 1% Phytohemagglutinin (PHA) and the epithelial cells were subcultured continuously to enable growth. metaphase chromosomes were collected at different cell divisions using Colcemide and Calyculin-A. Metaphase spreads were subject to fluorescence in situ hybridization (FISH) with whole chromosome probes for chromosomes 3 and 6 (MetaSystems). Chromosome aberrations were analyzed on Zeiss fluorescence microscope Axioplan 2 with Leica CytoVison software.

Femoral bone marrow single cell suspension was obtained from CBA/CaH and C57BL/6J male mice (8-14 weeks old). Cells were exposed to 600 MeV/u Fe ions (LET: 175 KeV/micron) and 600 MeV/u Protons (LET: 0.26 KeV/micron).

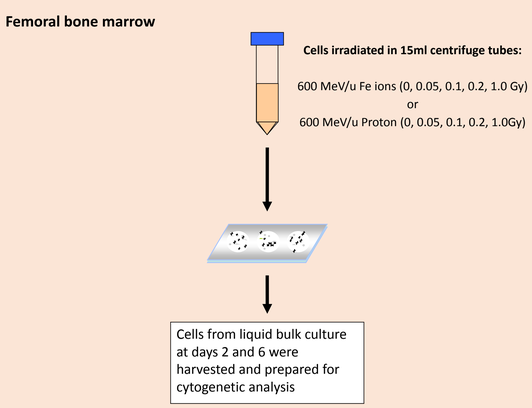

Figure 1. Radiation exposure of femoral bone marrow cells isolated from 8-14 week old male CBA and C57 mice.

FISH whole chromosome painting

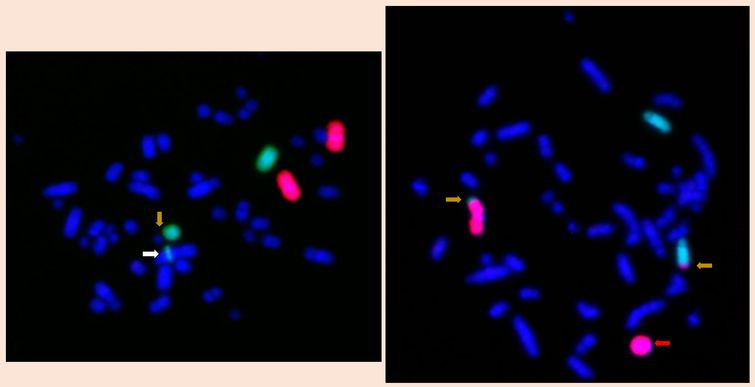

Figure 2. Examples of whole chromosome FISH (chr. 3, purple and chr. 6, azure) painting. Translocations are pointed by yellow arrows, insertions by white arrows, and ring by red arrow.

Results

Comparison of chromosomal aberrations in lymphocytes after Fe and Proton irradiation

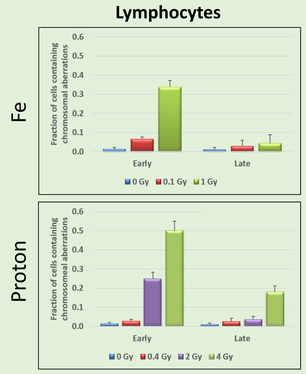

Figure 3: Different retention of chromosome aberration in lymphocytes after proton exposure at early and late time points. Data are fraction of aberrations in total counted metaphase spreads.

Chromosome aberrations in mouse bone marrow stems cells in liquid bulk cultures

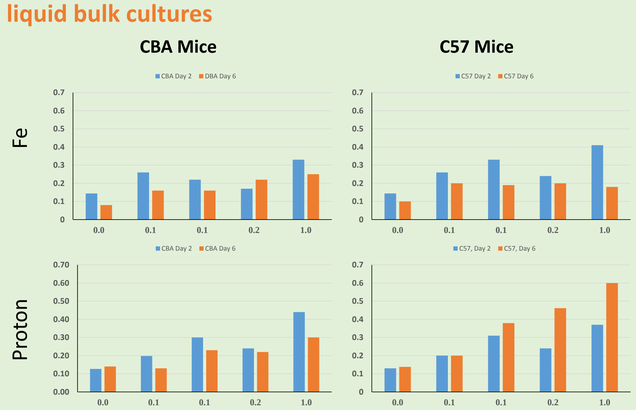

Figure 4. Transmissible chromosomal instability in liquid bulk cultures derived from Fe ion irradiated haemopoietic stem cells.

Results (Continued)

Radiation type dependence of genomic instability in early and late time points

Figure 5. Percentage of cells containing initial or late chromosome damage in human fibroblasts and epithelial cells after exposure to protons and Fe ions. Initial damage was assessed at first mitosis post irradiation, and the late damage was assessed at one month after exposure. Chromosome aberrations at one month post irradiation appeared to lack a clear dose response for the epithelial cells.

Conclusions

- In lymphocytes, the chromosome aberration frequency at 1 month after exposure to Fe ions was close to the unexposed background, whereas the chromosome aberration frequency at 1 month after exposure to protons was higher.
- Bone marrow cells isolated from CBA mice showed similar frequencies of chromosome aberrations between the early and late time points after proton or Fe ion irradiation, while cells from C57 mice showed different chromosome aberration rates between different time points.
- Mammary epithelial cells have a higher chromosome aberration background and higher rate of initial aberrations than the fibroblasts, but the fibroblasts retained more chromosomal aberrations after long term culture (1 month) in comparison to their initial damage.
- Caution must be taken in using RBE values to estimate health risks from space radiation exposure.

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